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14. ABSTRACT
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Report Title

Final Report - 2006 pathogen and toxin concentration systems for Water Monitoring

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easily be programmed to concentrate from 1 – 1000L.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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TOTAL:

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Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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Peer-Reviewed Conference Proceeding publications (other than abstracts):

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Graduate Students

NAME	PERCENT_SUPPORTED

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Names of Post Doctorates

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Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED

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Names of Under Graduate students supported

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Student Metrics

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The number of undergraduates funded by this agreement who graduated during this period:	0.00
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Total Number:

Names of other research staff

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Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See attached.

Technology Transfer

US Army Research Office Broad Agency Announcement
2006 pathogen and toxin concentration systems for Water Monitoring

Contract Number W911NF-06-R-0002

Design Review Report

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Udi Zuckerman, Co-PI, Tufts University
Greg Raih, Tufts University
Gary Stacey, VP Haemonetics Corporation
Bob Putt, Designer/Tech Writer Haemonetics Corporation

Executive Summary

In 2003, Tufts University developed a portable continuous flow centrifugation (CFC) device for concentrating *Cryptosporidium* oocysts from large water volumes using a Haemonetics Corporation disposable blood separation bowl. The methodology was validated by four independent water-testing laboratories and gained EPA approval as an alternate concentration method for *Cryptosporidium*. CFC has a significant advantage over conventional filtration; fouling or clogging is minimal, allowing much larger volumes to be processed. Additionally, the methodology is user friendly, rapid, robust and cost efficient.

Responding to the Department of Defense requests for improvements in the concentration of multiple waterborne pathogens, a proposal was submitted by both parties to design and construct a compact, portable automated device enabling the simultaneous concentration of protozoa, bacteria, bacterial spores, algae and viruses from large volumes of various matrices. The first prototype, named CFC100A, is a portable, self-contained device and the process of sample concentration and elution is completely automated. It can easily be programmed to concentrate from 1 – 1000L.

At approximately 30cm x 33cm x 40cm and 14kg it has a small footprint while housing a centrifuge, a high volume peristaltic pump and five high pressure pneumatic valves that are all controlled by a an onboard PLC computer system capable of storing numerous preset protocols. The PLC makes the machine versatile and flexible while greatly simplifying operation, which can be mastered with only a few minutes of training. It is easy to transport using a specifically designed cart that comes complete with a battery pack making the CFC100A a truly portable device.

Accompanying the CFC100A is an EnviroBowl Kit, a sterile, disposable set that consists of a modified HS Core Bowl, PVC tubing and two collection bags required for the concentrated sample's eluate. The disposable set is packaged separately in a tyvek sealed bag and is ETO sterilized.

Pathogens are concentrated inside the centrifuge bowl at 9000rpm. The water sample is driven through the centrifuge bowl at ~1.0 liter/min by the peristaltic pump, which results in the retention of larger particles such as protozoa, bacteria and bacterial spores, on the wall of the disposable plastic centrifuge bowl. Smaller particles such as virus, which escape the centrifugal forces are forced through a positively charged component fitted in the modified High Separation (HS) core of the bowl that adsorbs small negatively charged particles. The resulting concentrate from the automated concentration and elution procedure is small in volume and presentable to a wide variety of detection methods.

Preliminary testing showed >50% recoveries from small numbers of *C. parvum* oocysts, *B. anthracis* spores and MS2 bacteriophage when spiked into 10 and 50 liters of tap and source water samples. The device will be refined to increase the recoveries to >80%, decrease the concentrate volume as well as accommodate it in a single bag. The overall procedure will be validated in independent certified labs.

Table of Contents

SECTION 1: CABINET ENCLOSURE

The sheet metal enclosure, designed to house the electrical and mechanical components of the CFC100A consist of a base, top deck, rear panel, cover and display housing. All components are made of .09″ thick 5051 aluminum except the top cover and display housing which is made of.06″ thick 5051 aluminum. The enclosure is coated with a durable light gray epoxy powder-coat paint. Its design allows for easy component installation and assembly permitting quick and simple maintenance or repairs.

1.1. Enclosure Components

1.1.1 Base

The enclosure base has four rubber feet mounted to it at each corner fastened with an 8-32 sems screw and elevate the base approximately one inch. The centrifuge is mounted on the bottom of the base along with a bracket that secures a power supply, a 24volt solenoid, and a line filter. Two motor control units used to control centrifuge and pump speed are mounted on a separate bracket in front of the centrifuge.The base has two side frames used to mount the top deck and the rear panel with Pem inserts.

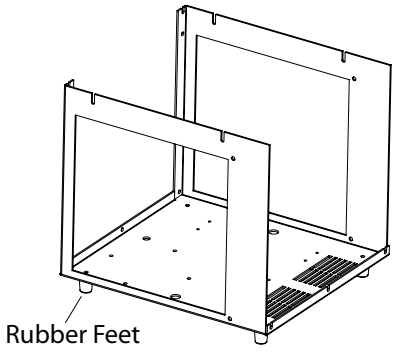


Figure 1.1

1.1.2 Top Deck

The top deck makes up the front and top of the enclosure. The pump is mounted to the top deck using three 8-32 cap screws. Five pinch valves are mounted on the top deck, two large valves and three small valves. The PLC control and the pneumatics module for the pinch valves are mounted on the front inside of the top deck. A labyrinth seal is placed around the centrifuge clearance hole and a seal is mounted at the back between the top deck and the top cover to protect against water leakage into the enclosure.

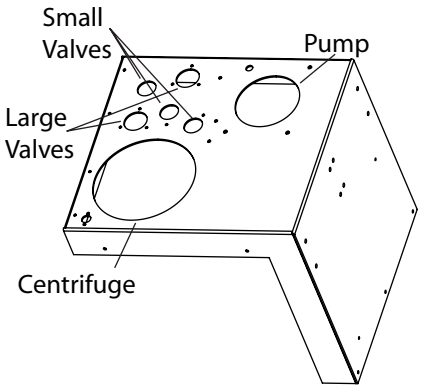


Figure 1.2

1.1.3 Rear Panel

The rear panel consists of three sides that complete the enclosures cabinet. A cooling fan, power entry module and recessed handles are mounted to the rear panel.

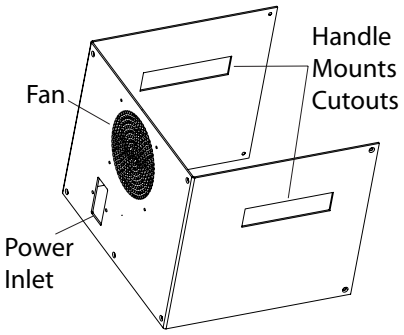
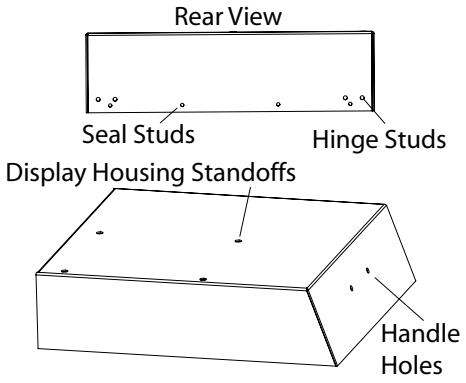


Figure 1.3

1.1.4 Top Cover

The top cover is hinged to the top deck by two friction hinges. A series of studs run below the hinge studs to secure a seal in the top deck to prevent fluids from leaking into the enclosure. An aluminum handle is mounted to the front of the top cover as well as the display housing.



1.1.4.1 Display Housing

The display housing is inserted into the top cover to house the PLC display. It is made up of two pieces that are retained to the top cover by standoffs.The studs in the display are retained using four keps nuts, which also fasten the top portion of the cover and insert.

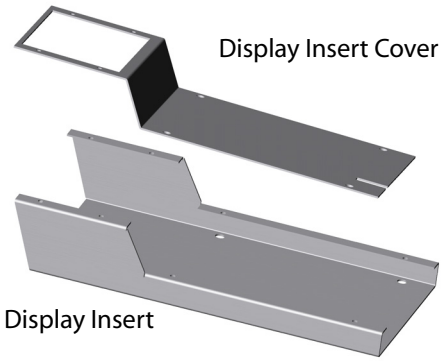


Figure 1.5

1.2. Shipping Container

The shipping container is made of 350T RSC Kraft material with internal dimensions of 16” x 17” x 21.5”. To protect the machine form damage, a 1.2 lb. polyethylene foam pads surrounds the internal compartment to secure the equipment.

SECTION 2: COMPONENTS

2.1 Pump

The pump used to control the direction and flow rates of fluids during the concentration and elution procedures. The pump rotor and platen assembly were designed for a high volume blood processing and have been used in the medical field for fifteen years. the pump has a high longevity and requires very little maintenance. Three pump rollers are spring loaded with three springs per roller and slide in a miter groove. The combined spring rating is sufficient for 3/8" tubing.

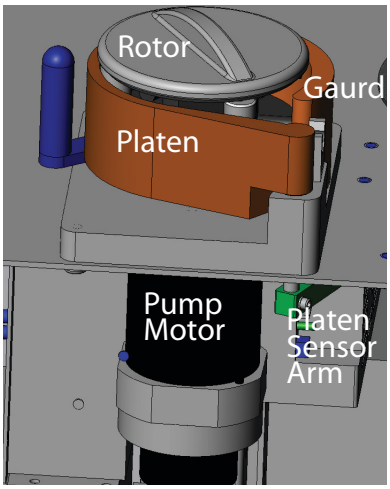


Figure 2.1

2.1.1 Pump Rotor

The rotor is driven by the pump motor using a mating square configuration between the motor shaft and the rotor. The motor shaft is fastened to the center of the rotor using a threaded cap.

2.1.2 Pump Platen

The pump platen pivots at one end in two nylon bushings to allow tubing to be inserted into the pump. A lever controls the pivot and, when closed, the lever pivots over-center to lock the platen in place.

2.1.3 Rotor Guard

The rotor guard is fixed to the base and encloses the rotor to protect from rotating parts.

2.1.4 Pump Position Sensor

Mounted under the pump base, on the platen shaft is the platen sensor arm. If the platen is opened when the pump is rotating, the pump will come to an abrupt stop.

2.1.5 Pump Motor

The pump motor is a brushless stepper to allow flow rates from 100 ml per minute down to zero.

2.2 Large Pneumatic Valve

The large pinch valve is made up of two parts, the valve assembly and the valve housing. They are retained to the underside of the top deck by studs and keps nuts.

2.2.1 Valve Assembly

The valve assembly is inserted into the valve housing and is retained with two screws and washers.An "O"-ring on the valve assembly that seals against fluids. Air pressure hods the valve open while spring pressure keeps it closed. The assembly has been tested for leak-down and has no leakage over five minute period.

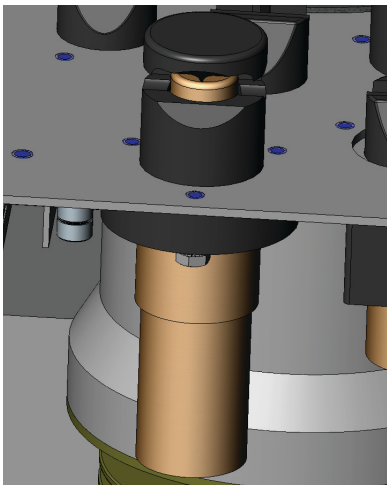


Figure 2.2

2.2.2 Valve Housing

The valve housing is custom designed for the CFC100A. IT is machined form 6061 aluminum and is black hard coated for durability.

2.3 Small Pneumatic Valve

The small pneumatic valve is a one piece assembly made by Areodyne Controls and has been used in various blood processing machines for over a decade with reliable results. They are secured to the underside of the top deck by studs and keps nuts. Air pressure holds the valve open while spring pressure keeps it closed.

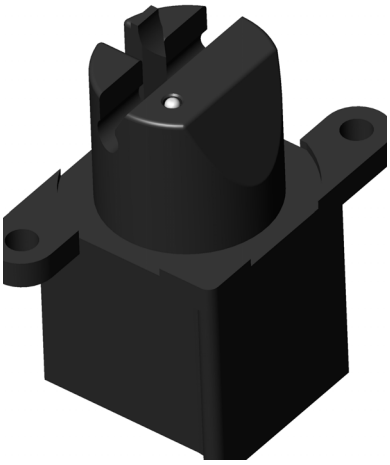


Figure 2.3

2.4 PLC

The DL06 PLC that electronically controls the machine is built by Automation Direct. It has 20 discrete inputs, 16 outputs and two built-in communication ports that are used for programming, operation interfaces or networking. One communication port is multi-functional capable of handling RS232C, RS422 or RS485 communication links. Over 220 different instructions are available for program development as well as extensive internal diagnostics that can be monitored form the application program or from an operator interface. Hand-held programers, operator interfaces, or a personal computer are easy to connect without needing any additional hardware.

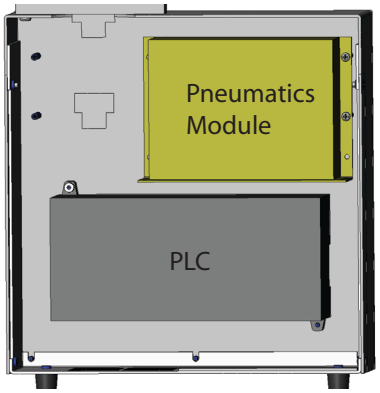


Figure 2.4

2.5 Pneumatics Module

The pneumatics module is a custom made unit that is used to supply air pressure to pneumatic valves in CFC100A. It has been used in the blood industry for over a decade with proven reliability.

2.6 Centrifuge

The centrifuge is manufactured by Magstar Corporation and is a fully enclosed unit. It is

capable of running from 0 to 10,000 RPM. It has a safety lock cover that can only be opened when the centrifuge has come to a complete stop.

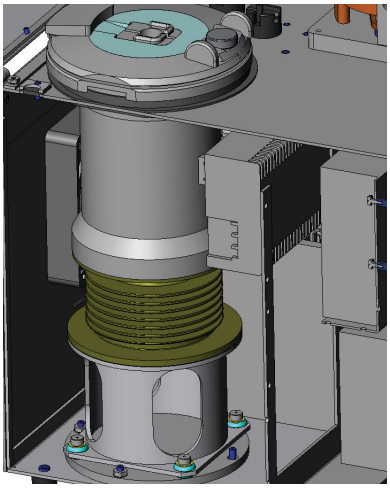


Figure 2.5

The centrifuge has a chuck that accommodates the HS Core blow-molded bowl that is lightly press in. The body of the centrifuge is mounted directly to the centrifuge motor housing. The motor specifications are as follows:

Table 1: Centrifuge Functional Specs

Function	Specification
Peak torque	480 oz-in
Current at cont tq	11.4 A
KE	5.0V/KRPM
KT	6.75 oz in/amp
Res Phase/Phase	.10 ohms
Inductance	.120 Mh
Rotation	Clockwise from top

The motor housing is mounted on a stand with four vibration isolators. The isolators sit on top of the centrifuge base and are secured with shoulder screws and brass washers. The base is secured to the cabinet base with four studs and four keps nuts.

2.7 Cooling Fan

The cooling fan is mounted on the inside of the rear panel. A series of holes drilled into the rear panel allow the fan to exchange air in order to keep the electrical components cool.

The fan's dimension are 4.69″ square by 1″ deep. It runs on 24 volts DC power and is UL certified. It draws 11 amps and is rated at 61 CFM.

Figure 2.6

2.8 Power Entry Module

The power entry module, manufactured by Interpower, is a three function single fuse unit mounted to the outside of the rear panel. The fuse is inserted behind a panel in the module for easy replacement. It has a current rating of 10A. Voltage rating is 120/250VAC and operating frequency of 50/60Hz.

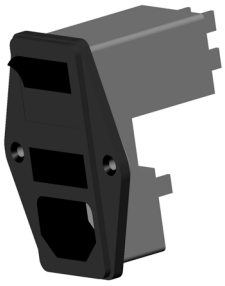


Figure 2.7

2.9 Recessed Handles

For lifting the CFC100A machine, two recessed handles are installed into the sides of the rear panel.

Figure 2.8

The handles are made of ABS and snap into the rear panel cutouts.

2.10 Motor Controllers

The centrifuge and pump motor controllers are Copley Controls Xenus XLJ-230-10. The Xenus XLJ-230-10 is a digital servoamplifier for brushless or brush motors used in stand-alone operation.

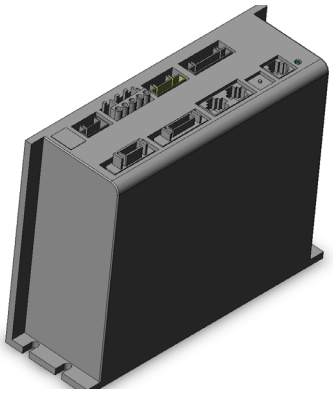


Figure 2.9

The key features of the Xenus XLJ-230-10 are:

Table 2: Xenus XLJ-230-10

Function	Specification
100 to 240 Vac Operation	Single or Three Phase
CANopen Distribution Drive	PTV, Profile, Homing Powerful Software Tools
Standalone Mode	Indexing ±10V Velocity/ Torque Command Electronic Gearing Hall or BEMF Velocity Control
Field -oriented Control for Optimal Speed/Torque	
Auto-tuning and Auto-phasing	
Motor Feedback	Digital Encoder and Halls Analog Sin/Cos Encoder Resolver versions

Table 2: Xenus XLJ-230-10

Function	Specification
Tri-Mode Encoder Port Programable as:	Dual Encoder Input, or Buffered Motor Encoder Output. or Emulated Encoder Output from Sin/Cos
Programmable I/O	12 inputs. 4 outputs
Opto-isolated Brake Output	

2.11 Power Supply

The CFC100A is powered by an Astrodyne MK75S-24 which has an input Voltage range of 90-264 VAC and an output voltage of 24 VDC.

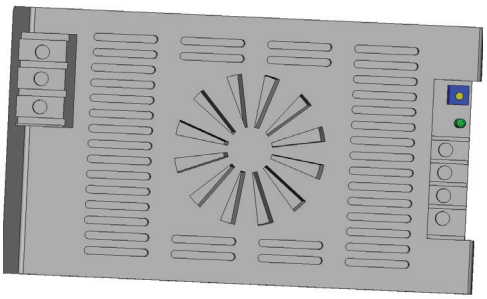


Figure 2.10

2.12 Relay

The 70-782EL8-1 socket relay, manufactured by Magnacraft, is an eight pin logic style DIN panel mount with elevator terminal, module compatible relay. It has a normal voltage rating of 300 volts and normal current rating of 12 amps. The relay is used for commutation between the PLC and the motor controllers.

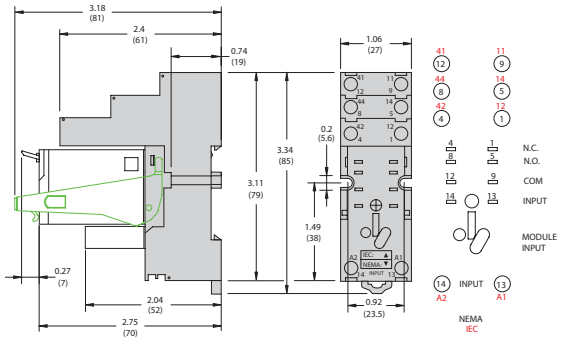


Figure 2.11

The SF20L filter by Filter Concepts is designed to effectively attenuate differential (L/L) and common mode (L/G) noise from switch mode power conversion and regulation circuits subject to FCC Class B or CISPR//EN Class A conducted EMI limits. It has a current rating of 20 A and a leakage specification of.80 mA.

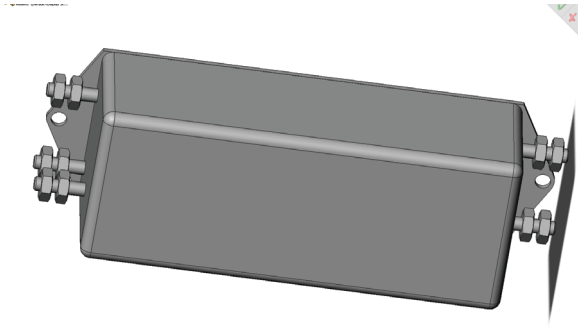


Figure 2.12

The filter provides maximum attenuation form 150 KHz and above. The filter is also available with the “A” circuit option to enhance its performance in applications with high source impedance.

SECTION 3: DISPOSABLE SET

3.1 Introduction

The disposable set is used to carry the water sample and elution buffers to the centrifuge for concentration and solution of concentrate. The set is comprised of large diameter PVC tubing, small diameter PVC tubing, a modified HS Core bowl and two PVC bags to hold the elution buffers and receive the concentrate eluate. Large diameter tubing is attached to the inlet and outlet ports of the modified HS Core bowl, which is held in the centrifuge chuck. The pump drives the water sample through the large diameter tubing to the inlet port of the bowl. Waste water is carried through large diameter tubing from the outlet port of the bowl. During the elution process the small tubing carries the concentrate eluate back to the bags when the elution cycle concludes. The set is coupled by a variety of PVC connectors.

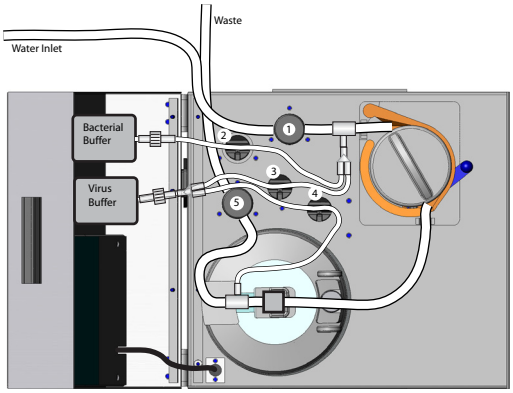


Figure 3.1

3.2 Modified HS Core Bowl

The High Separation Core Bowl ia manufactured by Haemonetics Corporation. The core is custom modified for this unique application with the addition of positively charged material and a membrane welded to the top to retain the material.

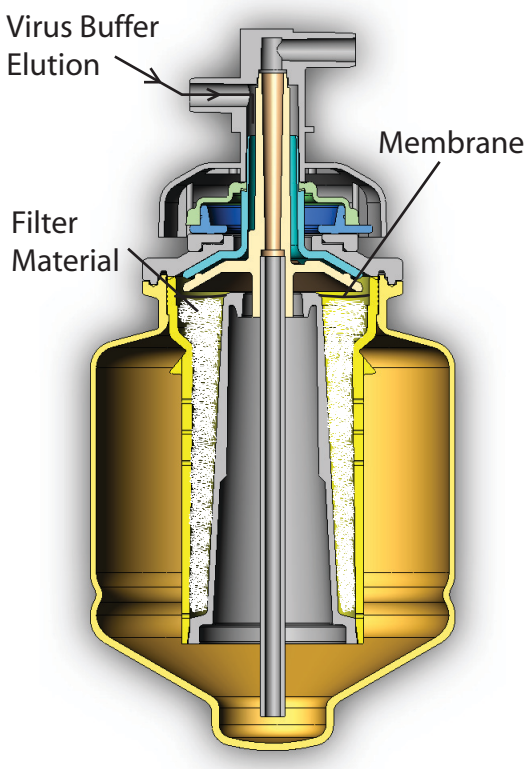


Figure 3.3

3.3 Bacterial/Protozoa Elution Buffer

The bacterial/protozoa elution buffer is a 5xPBS and 0.01% Tween 80 solution used as a detergent to dislodge bacteria and protozoa that have compacted to the walls of the bowl during the concentration process.

3.4 Virus Elution Buffer

The virus elution buffer is a beef extract, glycine and Tween 80 solution used to neutralize the positive charge of the positive material inside of the core and dislodge the virus that were adhered during the concentration process.

3.5 Large Tubing

The large tubing, manufactured by Navatar Co. Filtrona Extrusion Inc., is made of clear PVC resin. Its has a durometer of 62/68 shore "A". The inner diameter i

250″ ±.005″ and and an outer diameter of .375″ ±.005″. It is certified to be free from pyrogens, dirt, oils, and imperfections.The raw material used to make the tubing must meet biocompatability requirements stated in Haemonetics' QCPA.03.

3.6 Small Tubing

The small tubing, manufactured by Navatar Co. Filtrona Extrusion Inc., is made of clear PVC resin. Its has a durometer of 65/75 shore "A". The inner diameter is .120″ ±.003″ and and an outer diameter of .170″ ±.003″. It is certified to be free from pyrogens, dirt, oils, and imperfections.The raw material used to make the tubing must meet biocompatability requirements stated in Haemonetics' QCPA.03.

3.7 Tubing Connectors

The disposable set uses two types of tubing connectors to couple the set together. The large tubing is joined by a "T" connector. Three pieces of small tubing or small tubing and large tubing are joined by a "Y" connector. All connectors are supplied by VitalMed Inc. which have been contracted to manufacture the disposable set.

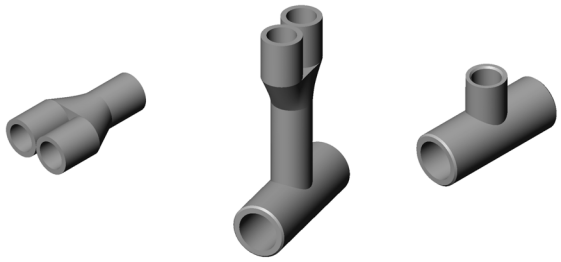


Figure 3.4

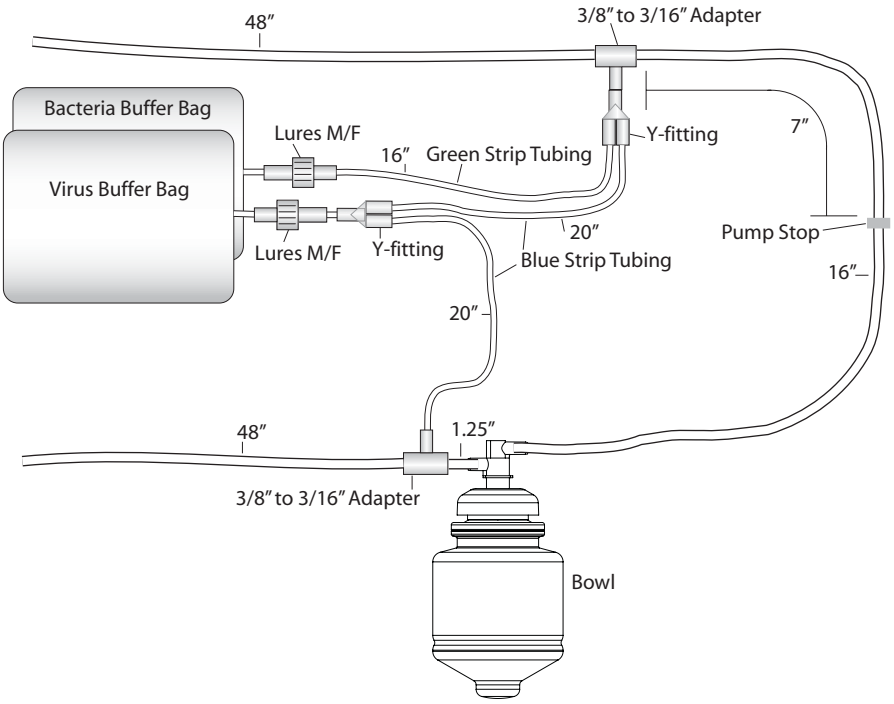


Figure 3.5 Disposable kit

SECTION 4: OPERATION PROCEDURE

Starting the CFC100A

1. Take the power cord, supplied with the CFC100A, and install it into the power entry module at the rear panel of the machine.

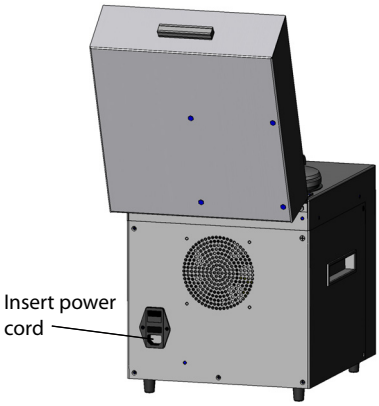


Figure 4.1

2. Take the free end of the power cord and install it into an AC outlet.

Note: The design plan calls for running the CFC100A by battery power. The batteries will be stored in a transport cart that will mount to the CFC100A. See Appendix A for details.

3. Press the switch on the power entry module to the “ON” position.

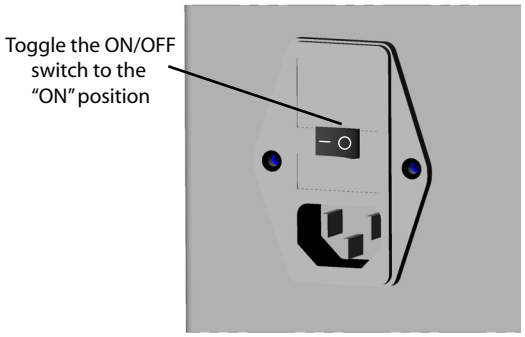


Figure 4.2

Preparing the CFC100A

4. Once the machine is powered “ON” the display panel will read “Press Select”. Press the select button on the display which will open all the pneumatic pinch valves.

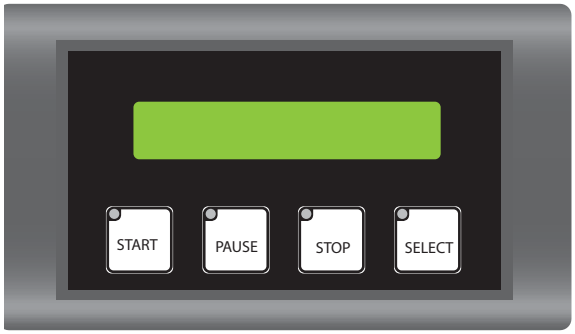


Figure 4.3

5. On the centrifuge cover, turn the knob at the front to the unlocked position and push down and lift the cover open.

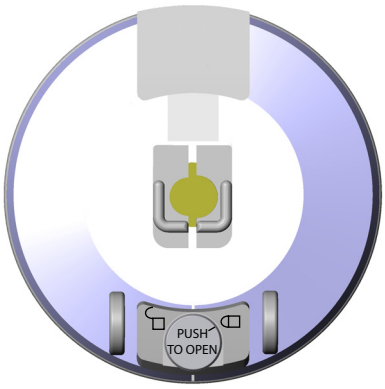


Figure 4.4

6. On the pump, pivot the platen lever arm to the open position.

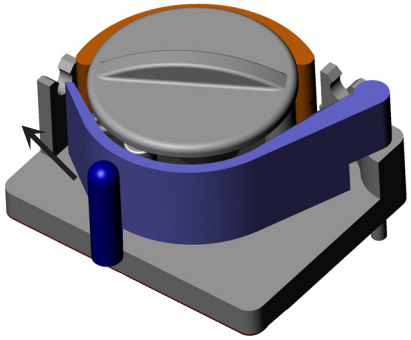


Figure 4.5

Elution Buffers

7. Remove a new, sterile harness form its packaging.

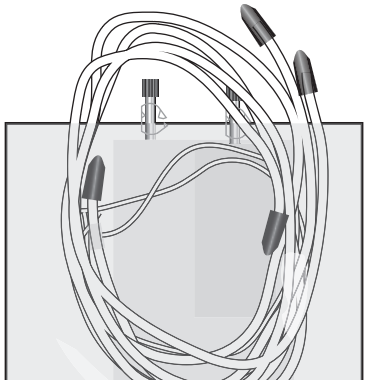


Figure 4.6

8. Elution buffers are supplied in separate syringes labeled as virus buffer (20mL) and protozoa/bacteria buffer (5mL).

9. Inject the virus buffer into the 100 mL virus bag by detaching the lure lock from the harness and locking the syringe to the bag and injecting the contents. Close the clamp on the bag and reattach the bag to the disposable set.

10. Inject the protozoa/bacteria buffer into the 500 mL protozoa/bacteria bag by detaching the lure lock from the harness and locking the syringe to the bag and injecting the contents. Close the clamp on the bag and reattach the bag to the disposable set.

Loading the Disposable Set

11. Remove a new sterile Multiple Pathogen Bowl from the bulk pack and install it into the centrifuge chuck. Ensure that the inlet port (highest) is facing the front of the machine.

Caution: When installing the bowl into the centrifuge chuck, push firmly on the bowl to seat it to the bottom of the chuck.

12. Close the centrifuge cover and turn the knob to the locked position.

13. Install the inlet tube from harness to the pump, noting the pump stops locate to the outside of the pump stop supports on the pump.

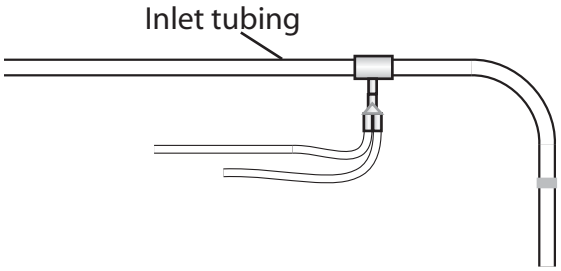


Figure 4.7

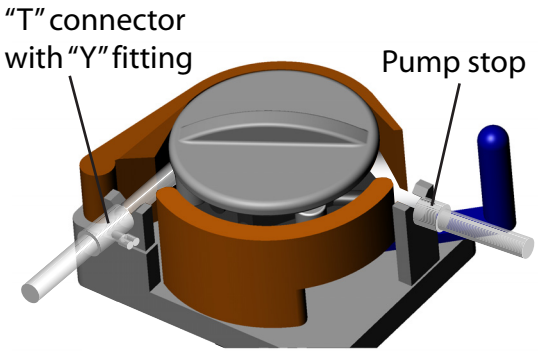


Figure 4.8

14. Close the platen lever to the pump to clamp the inlet tubing.

15. Connect the lose end of the inlet tubing to the inlet port of the bowl assuring that the inlet tube covers the entire port.

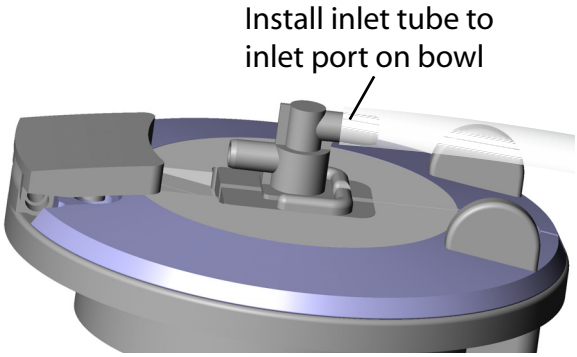


Figure 4.9

16. Install the inlet line into pinch valve number one. See Figure 4.11.

17. Install the waste tube from the harness to the outlet port of the bowl. Ensure that the tube covers the entire port.

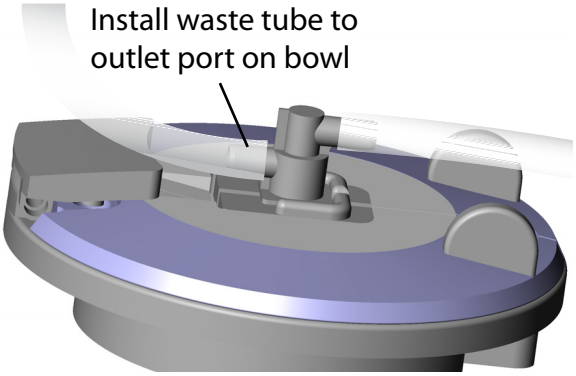


Figure 4.10

18. Install the waste tube into pinch valve number five. See Figure 4.11

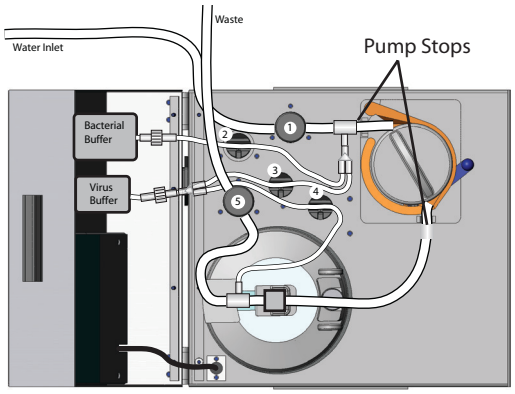


Figure 4.11

19. Hang the bags containing the solution buffers on the hooks at the top of the machine's cover.

20. Insert the green striped tubing into pneumatic pinch valve number 2. See Figure 4.11

21. Install the two blue stripped tubes into pneumatic pinch valves 3 and 4.

22. The blue line from the "Y" connector at the pump goes into pinch valve 3 and the blue

line from the outlet port of the bowl goes into 4.

23. Press the select button on the display to close all the pneumatic valves. Open the slide clamps on the tube of each bag containing the elution buffers.

Beginning the Concentration

24. Place the inlet tube into the water supply.

25. Place the outlet (waste) tube into a waste container. The outlet tube should not be submerged so waste is not returned to the bowl during the solution process.

26. Press start on the display panel to begin the automated concentration and solution of the water sample.

Removing Eluates

27. Once the entire concentration and solution process has finished the display panel will read “Cycle Complete”.

28. Close the slide clamps on each bag and detach them form the rest of the disposable set and keep for analysis.

29. Press select on the display panel to open all the pneumatic pinch valves and discard the rest of the set.

Decontamination

There is no need to decontaminate the equipment since it uses a new disposable concentration set for each cycle.

SECTION 5: MODIFIED HIGH SEPARATION BOWL

5.1 Disposable Bowl

A modified disposable bowl was designed and constructed at Tufts University. The parts used to construct the bowl were from the standard High Separation Core Bowl, manufactured by Haemonetics. It has a unique two-piece core that was modified with a total of 24 holes (2mm in diameter) drilled around the lower perimeter of the HS Core at a 45^{o} angle from each prior set. The six original holes in the upper half of the HS core were sealed with hot glue. In addition, positively charged material was added to the outflow chamber of the core for the capture of viruses.

5.2 Positively Charged Material

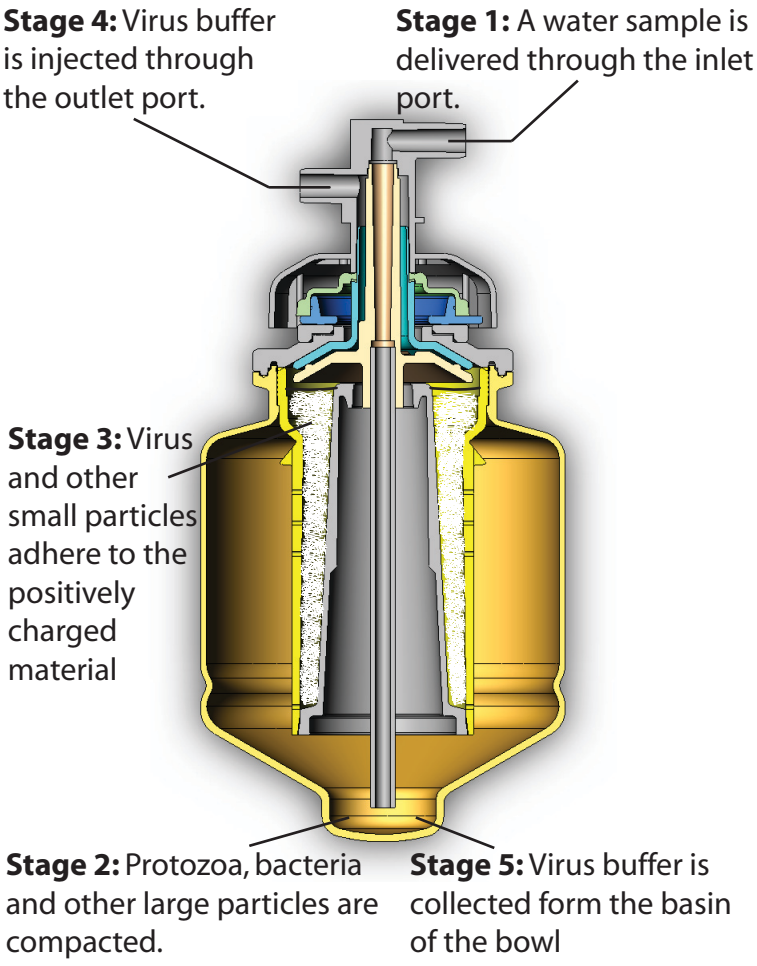
The configuration consists of 1g Aluminum Hydroxide, $Al(OH)_3$, nano-ceramic fibers Boehmite (Argonide Co. R0608) and 3g paper pulp per core packed in the outflow chamber to form a consistent, dense, but fluffy matrix. A Hollytex 3267 spun-bound polyester fiber cover support (Ahlstrom Technical Specialties 50996-00) was cut to fit and seal the top of the outflow chamber, securing the material within.

After preparing the cores at Tufts University they were sealed and assembled in bowls at Haemonetics.

5.3 Concentration Process

During the concentration process heavier particles, protozoa, bacteria and other organic and non-organic particles, are compacted to the walls of the bowl by the centrifugal forces. Cleaner water flows through the designated passages into the inner core and through the positively charged material. Smaller particles that have escaped the centrifugal forces, such as virus, are adsorbed to the positively charged material. The modification of the inner core enables pathogens of all sizes to be concentrated in one process and subjected to an automated elution cycle. To elute virus from the inner core virus elution buffer is injected through the outlet port to saturate the charged material within. The centrifuge spins and the centrifugal force extracts the buffer through the water passages of the core in the bottom of the bowl. The resulting eluate is then pumped into a designated bag through the inlet port.

5.4 Modified High Separation Core Bowl

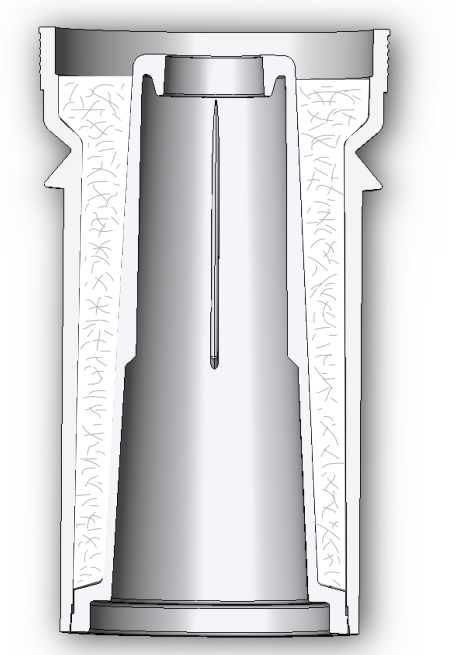


5.5 Water Passages

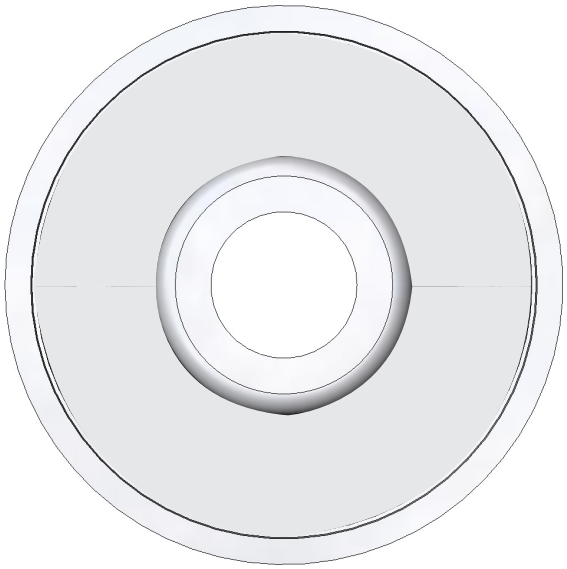
The top six water passages in the original HS Core are sealed.

New passages (6 sets of 3) are drilled in the bottom to allow a homogenous water flow through the charged material.

5.6 Charged Material

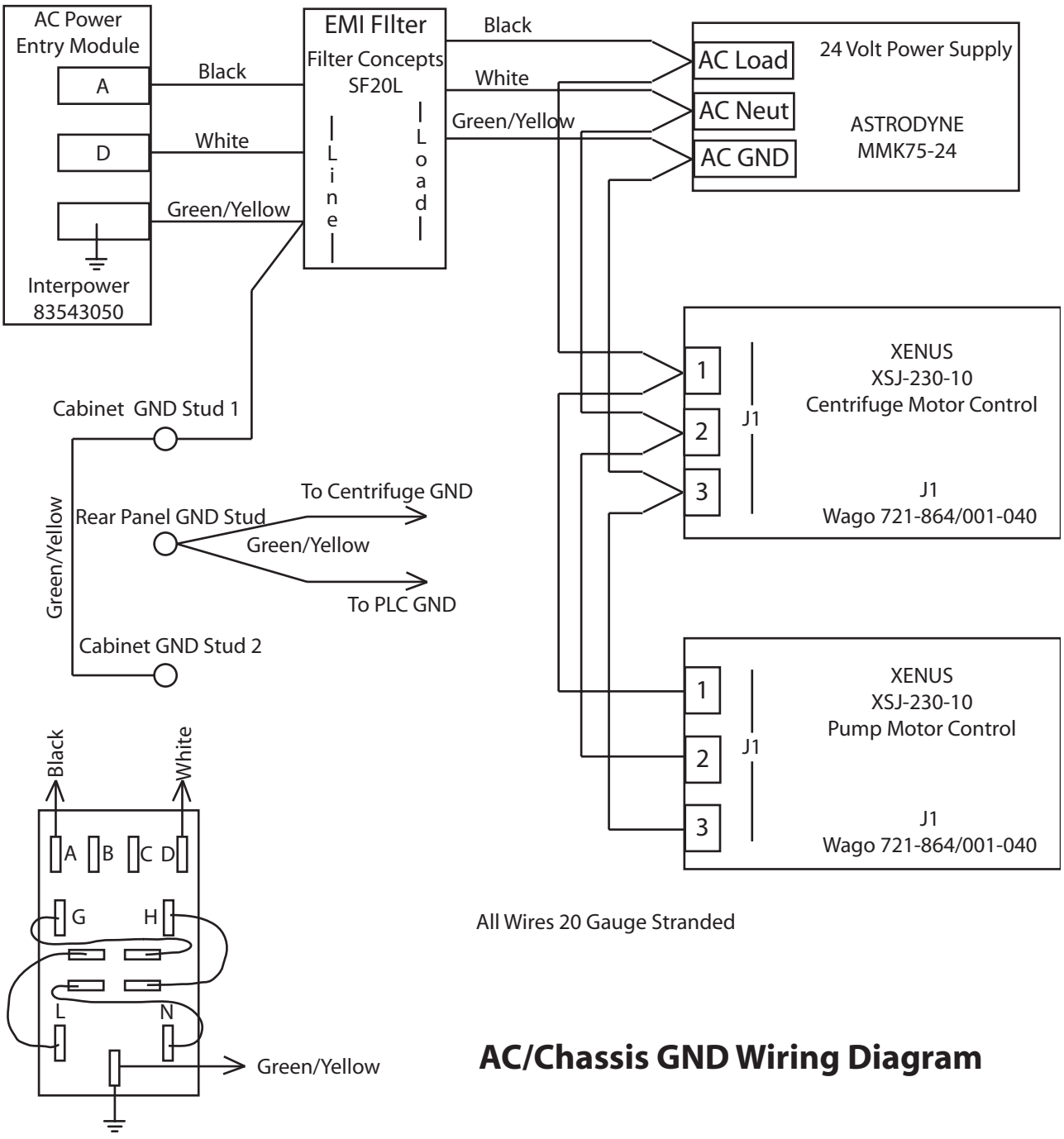


5.7 Cover Support



SECTION 6: WIRING DIAGRAMS

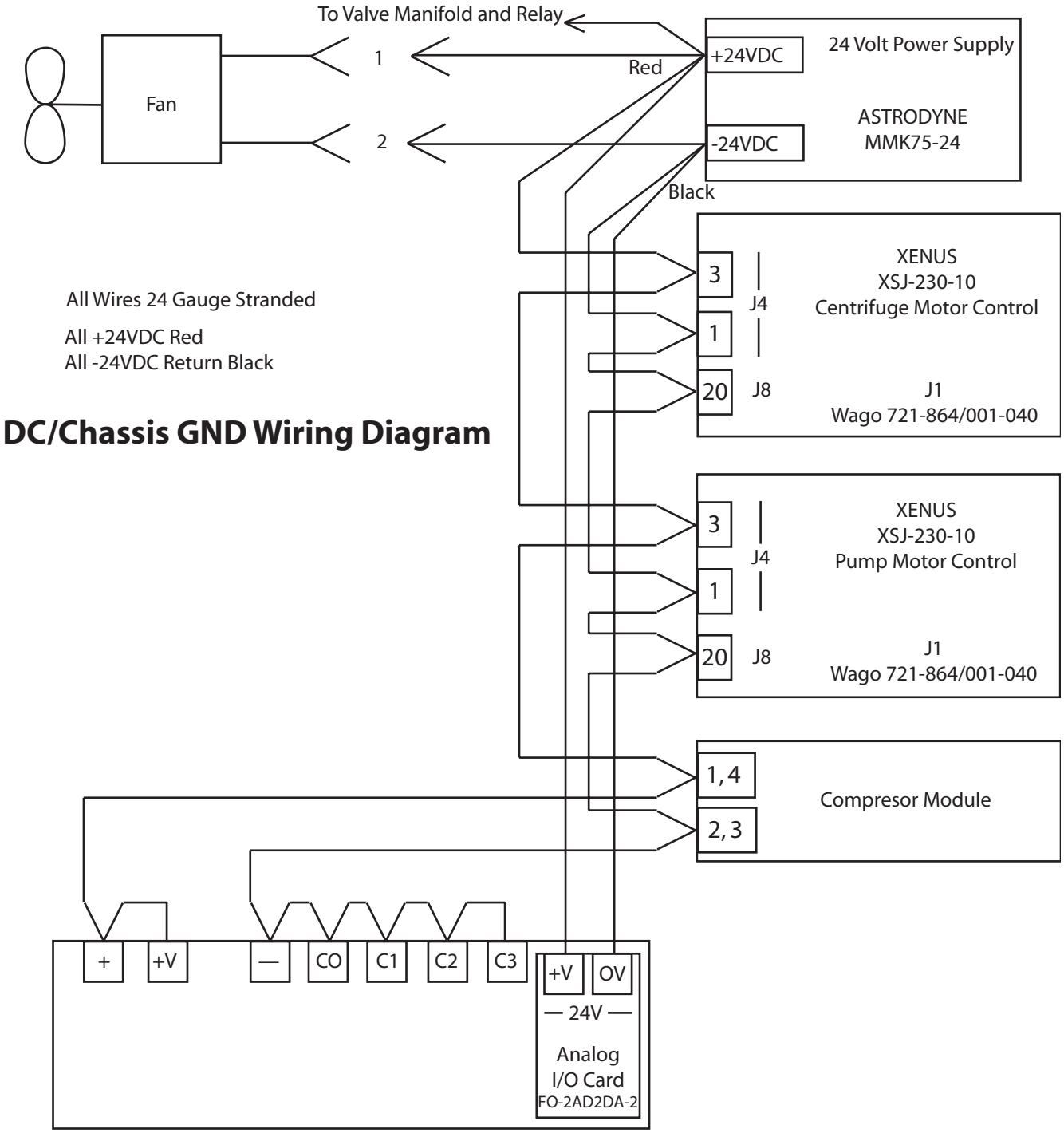
6.1 AC Wiring Diagram



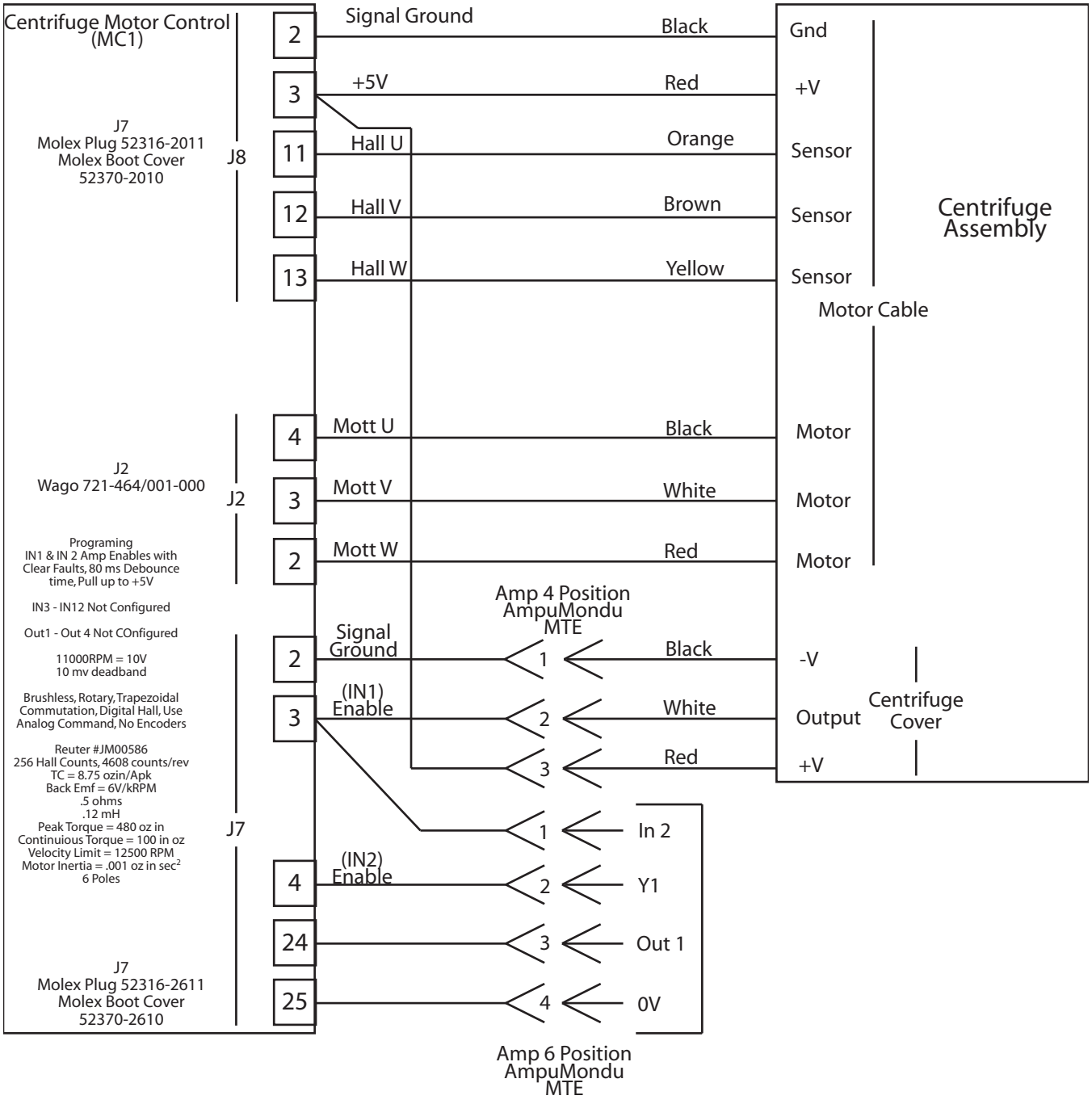
All Wires 20 Gauge Stranded

AC/Chassis GND Wiring Diagram

6.2 DC Wiring Diagram

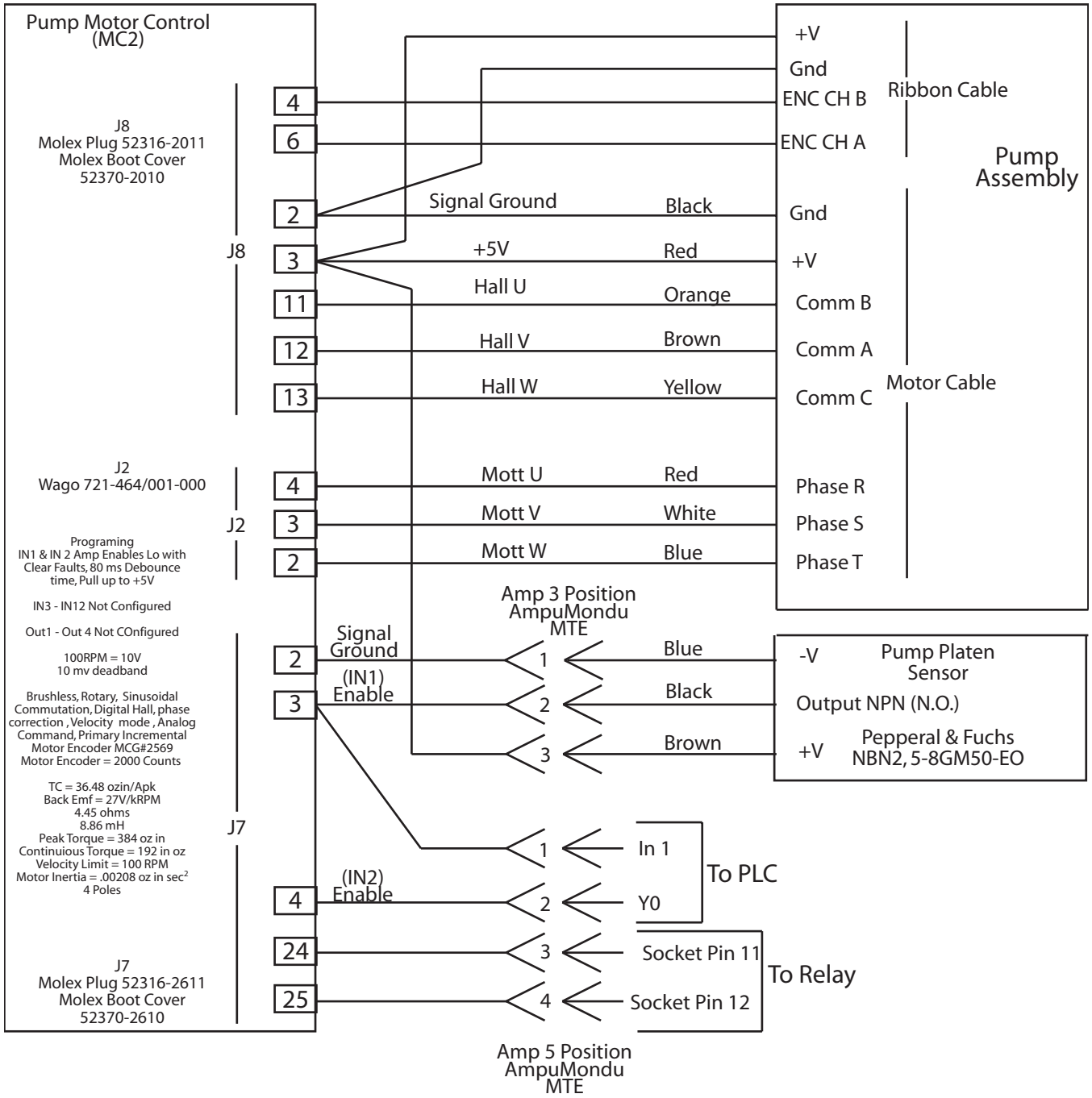


6.3 Centrifuge Wiring Diagram



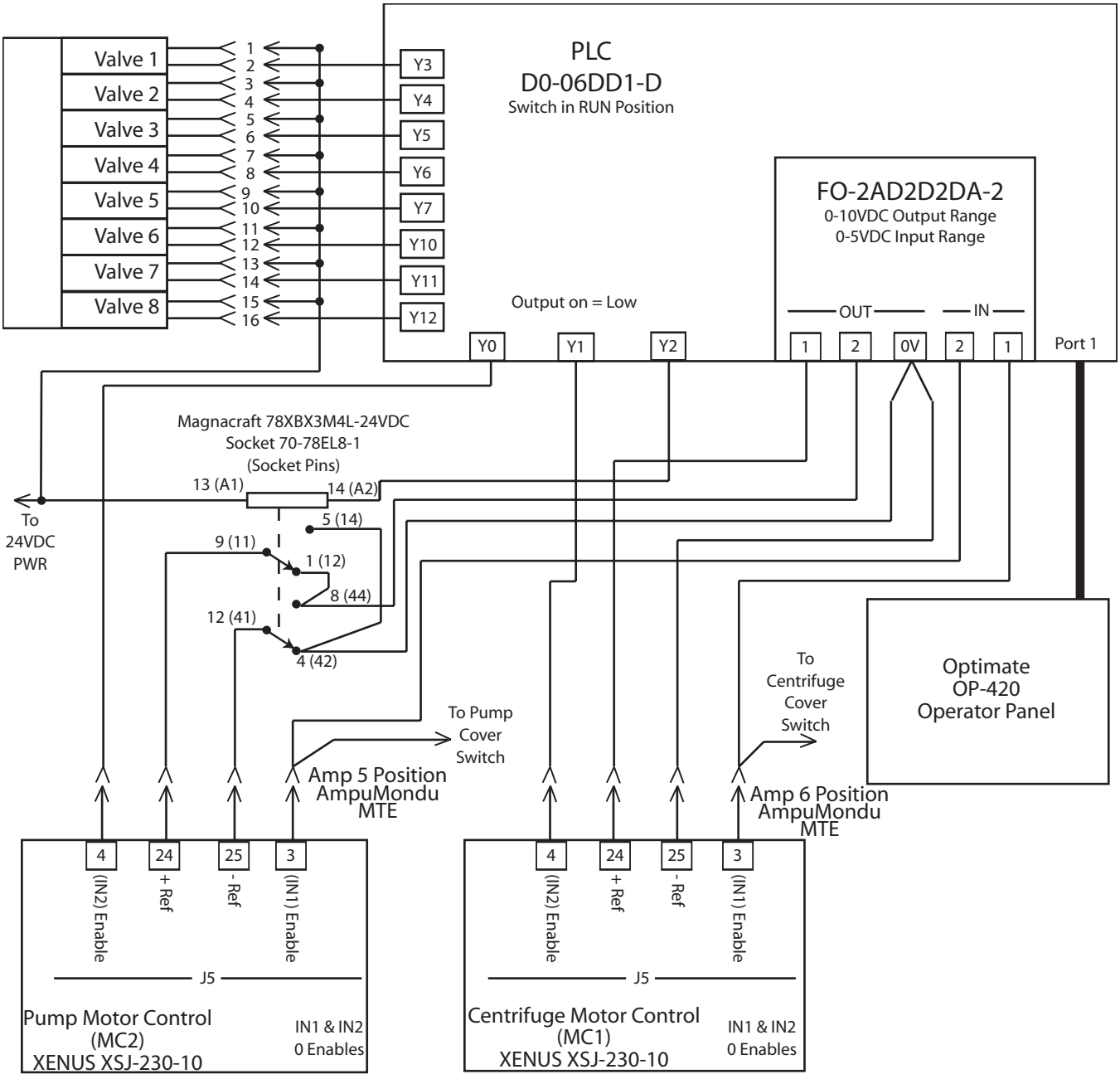
Centrifuge Wiring Diagram

6.4 Pump Wiring Diagram



Pump Wiring Diagram

6.5 Control Wiring Diagram



Note 1:
Centrifuge Cover Switch and Pump Cover Switch
Pin 3 on Motor Controls/ In 1 & 2 on PLC
0 = Closed and OK to Run
5 Volts = Open and Halt

Note 2:
Pin 4 on Motor Controls
Y0 & Y1 on PLC
PLC Output ON = 0 = Run

Control Wiring Diagram

APPENDIX A

Transport Cart

The design plan calls for a transport cart that will contain storage space and a battery pack for operating the CFC100A remotely from a wall power source. The machine will be able to run on 12 or 24 volt battery power that is run through a converter.

The CFC100A is mounted to the top of the cart with two inserted fasteners on the sides. Once the machine and cart are fastened together, it can be transported over most terrain by the use of large cushioned rear wheels.